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COPPER AND SILVER-BASED NATURAL HYDROGELS: ADVANCING WATER RETENTION AND CROP PRODUCTIVITY IN AGRICULTURE

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ABSTRACT
India, a major hub of agricultural activity, supports a significant portion of its population through farming and related occupations. However, the growing population has driven up the demand for food, prompting farmers to rely increasingly on chemical fertilizers, pesticides, and other synthetic inputs to boost crop yields.Although these chemicals increase the yield, they also contaminate our environment and have adverse effects on human health. Keeping this in view, in the present work, an attempt has been made to fabricate natural hydrogels using nanotechnology, which are safe for the environment. These hydrogels can not only satisfy the farmers but also encourage sustainable farming. The fabricated hydrogels have been synthesized through chitosan-based silver and copper nanoparticles using leaf extracts of Neem (*Azadirachtaindica)*, Giloy(*Tinosporacordifolia*), and Bryophyllum(*Kalanchoe pinnata*). The effect of these nanoparticles has been studied on the seeds of *Vigna radiata,* and various parameters such as root length, shoot length, surface area of leaves, water consumption, etc., have been studied. The results of this study suggest that the growth is better in the treated plants and less amount of water is required by them as compared to the control.
Keywords: Agriculture, Chitosan, Nanoparticles, Sustainable, Water Stress.
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INTRODUCTION

Agriculture plays an essential role in ensuring food security and fueling economic development. In India, approximately 70% of the population depends on agriculture and related sectors for their means of livelihood.It contributes up to 13.9% of India's gross domestic product (GDP) and 28% of our total GDP, and generates employment for 54.6% of the population.[1] Since the last century, the increasing worldwide population has been demanding more food and resources.Quantitative scenario analyses suggestthat, depending on various socio-economic and climate conditions, global food demand is projected to increase by approximately 35% to 56% between 2010 and 2050.[2]Due to this, the pressure has come to the agriculture sector, leading to overuse of chemicals as fertilizers and pesticides. Synthetic fertilizers are being used uncontrollably to fulfill the demand, escalating the environmental issues such as loss of biodiversity, climate change, soil degradation, etc.[3]The water scarcity also directly affects the agricultural sector. Climate change is another reason that the agricultural system is under stress. Crops are consistently exposed to abiotic and biotic stress factors, which significantly hinder their growth and reduce overall yield. The simultaneous presence of abiotic stresses such as drought and heat waves poses a serious threat to crop productivity.[4] An alternative to overcome these stresses is the use of hydrogels in agricultural land or hydroponics.Sustainable agriculture strikes a balance between need and supply, along with the preservation of the ecosystem. Over the last 40 years, the agricultural sector has been focusing on hydrogels to take a step towards sustainability, as they enhance water availability for crops by increasing the water-holding capacity of soil.[5] Hydrogels are three-dimensional cross-linked hydrophilic polymeric networks, having the capability to hold and absorb large quantities of fluids. They can be classified based on cross-linking, physical appearance, charge on the chain, configuration, and polymeric composition. They possess elastic networks with interstitial spaces capable of retaining up to 90–99% (w/w) water. The

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physical stimuli, like pressure, light intensity, temperature, electrical and magnetic fields, and chemical stimuli like solvent composition, pH, cause sol-gel transitions, which are reversible in nature in most cases and hence allow swelling and shrinking according to their surrounding conditions.[6] Hydrogels have high absorptive capacity and can absorb solvents from 10% to 1000 timestheir dry weight.[7] Hydrogels have wider applications because of their non-toxicity, low cost, and environment-friendly nature. Hydrogels are especially effective in agricultural settings with poor irrigation and sloped terrain vulnerable to runoff and erosion.[8] Hydrogels are also useful in daily life, and their major applications are in food, medical treatments, agriculture, industry, cosmetics, and medicine.Hydroponics is a beneficial, sustainable agriculture practice where plants are continuously supplied with a circulating mineral-rich nutrient solution, and have no risk of biotic or abiotic stresses as they are grown in controlled conditions. From an economic point of view, it also holds the potential to create opportunities for enthusiastic entrepreneurs in the form of urban and suburban container farming, thus creating specific local markets for fresh organic produce. Interestingly, the growth rate of a hydroponic system can be 30% more than a traditional soil-based system.[9]Chitosan has a high affinity for Cu (II) as compared to other metals, allows abstention of high encapsulation efficiency, and also helps in boosting the defence response against pathogenic fungi.[10] Chitosan is the most abundant natural amino polysaccharide, which is derived from the deacetylation of chitin found in crustaceans' shells, insects' cuticle, and the cell wall of various fungi.[11] It is widely used in the preparation of hydrogels due to the presence of hydrophilic functional groups and consists of two subunits, i.e., D-glucosamine and N-acetyl-D-glucosamine, which are linked together by beta-1,4-glycosidic bonds with two functional groups, i.e., free amino group at C-2 and hydroxyl groups at C-3 and C-6.The present study is focused on the development of innovative natural material-based systems that involve environmentally sustainable materials like chitosan and use of fabrication, i.e., Cross-linking of hydrogel with hydrophilic groups was facilitated to increase water absorption capacity, and provide stability after swelling and during storage, non-toxicity, and wettability. Here, the chitosan was cross-linked with leaf extract of Bryophyllum (*Kalanchoepinnata*), Neem *(Azadirachtaindica*), and Giloy (*Tinosporacordifolia*), and using this chitosan leaf extract complex, nanoparticles of silver (Ag NPs) and copper (Cu NPs) were prepared. The extracts of these plants are known to have antimicrobial properties and are known to be antibacterial, antiviral, antioxidant, and antifungal agents.[12] Coating of Mung bean seeds with these nanoparticles was done, and then the seeds were grown in soil, hydroponics, and a germination sheet for further studies to be carried out.

EXPERIMENTAL

Materials Required

Fresh leaves of Neem (*Azadirachtaindica*), Giloy (*Tinosporacordifolia*), and Bryophyllum (*Kalanchoe* pinnata) were collected from the Maitreyi College Garden in New Delhi. Mung beans (Vigna radiata) were acquired from a local store in New Delhi. Silver nitrate ($AgNO_3$) and copper nitrate ($CuNO_3$) from CDH, along with chitosan, sodium hydroxide (NaOH), and acetic acid, were also included in the study.

Sample Collection

We carefully collected the fresh leaves of Neem, GiloyandBryophyllum directly from their respective trees. We washed the leaves to ensure they were free from any impurities. After that, we air-dried them under a shed until they dried. Following this drying process, we ground the leaves into a fine powder using a grinder.

Preparation of Extract

Measure 1g of powdered Neem leaves, Giloy, and Bryophyllum each. Add them to 100 mL of water in a beaker. Heat the mixture on a burner and boil it until the liquid reduces to half of its original volume. After boiling, let the mixture cool down to room temperature. Filter the solution to remove any solid particles or residue.

Synthesis of Silver and Copper Nanoparticles

AgNPs and CuNPs were synthesized by the reduction of $AgNO_3$ and $CuNO_3$ with freshly prepared leaf extracts of *Antelaeaazadirachta*, *Tinosporacordifolia*, and*Kalanchoe pinnata.*10 mL of 1mM $AgNO_3$/ 3 mM $CuNO_3$ solution was reduced by 5 mL of leaf extract and 10 mL Chitosan solution (1 g of chitosan was dissolved in 100 mL of 1% acetic acid to prepare the solution.) with continuous stirring at 45°C, and further subjected the blend to microwave irradiation for 30 seconds at 720 watts. As a result, the color change of the solution to dark yellowish brown indicated the bio-reduction of Ag^{2+} to Ag, which was monitored by UV spectroscopy. The nanoparticles suspension (NAC–*Azadirachtaindica*+ AgNO3 solution + Chitosan, BAC - *Kalanchoepinnata* +$AgNO_3$ solution + Chitosan, GAC- *Tinosporacardifolia* +AgNO3 solution + Chitosan, NCC- *Azadirachta*indica + $CuNO_3$ Solution + Chitosan, BCC- *Kalanchoepinnata* + CuNO3 Solution + Chitosan, GCC – *Tinosporacardifolia*+ $CuNO_3$ Solution + Chitosan) was centrifuged and washed thrice with deionised water at 20,000 rpm.

Germination of Seedlings

Vigna radiata seeds were treated with synthesised silver and copper nanoparticles, prepared by using different leaf extracts [like NAC, BAC, GAC, NCC, BCC, GCC] *, by suspending in a 30 mL solution at 2500 ppm. 90 mung bean seeds were soaked in each solution for 8 hours. Also, 90 mung seeds were soaked in tap water as a control. Seeds were soaked to study the germination percentage and seedling growth. To study these parameters, seeds were sown into soil and hydroponics. The germination studies were carried out on the germination sheets placed in petri plates. The control was prepared for each set of experiments. After 24 hours, the set-ups were observed for germination.

Morphological Parameters

The amount of water sprinkled on the experimental set-ups was measured every day. After 48 hours and subsequently after every 3 days, the seedlings were analysed for various morphological parameters such as number of leaves, number of branches, root length, shoot length, and length of cotyledons. The growth parameters were measured for 10 days in the Hydroponics and Soil setups. After a span of 15 days, the plants cultivated in the soil were carefully uprooted to quantify the length of their roots. Also, on germination, seed only percent germination and root length were observed for 6 days.

Phytotoxicity Index

The toxicity in the seedlings was determined by calculating the germination index using the following formula, G_{index}= ((G/Go) × (L/Lo)) × 100, "G" and "Go" here represents the quantity of germinated seeds in the NPs treated experimental arrangement and control experimental arrangement, respectively, while "L" and "Lo" represents root lengths of seedlings in the NPs treated experimental arrangement and control experimental arrangement, respectively.

RESULTS AND DISCUSSION

UV/Visible Spectrum

Synthesised Ag and Cu nanoparticles were characterized by a UV-Visible spectrophotometer (Fig.-1). The peaks observed from 400nm to 420 nm in UV-visible spectroscopy confirm the presence of silver nanoparticles (Fig.-1a).[13] Maximum absorbance for Cu Nanoparticles was observed in the range 500 nm to 600 nm, hence confirming the presence of copper nanoparticles.[14]

X-Ray Diffraction

XRD patterns of copper nanoparticles and silver nanoparticles synthesized by using chitosan and leaf extracts of Neem, Giloy, and Bryophyllum are monitored at a high angle of 2θ range (0°-100°) as shown inFig.-2). The XRD pattern shows a crystallinity of the Cu sample level with various diffraction angles as given in literature (Fig.-2b).[15] There are peaks at 43.2°,50.4° and 74.10°, confirming the formation of copper nanoparticles[16.] In the case of GAC nanoparticles (Fig.-2a). There are peaks at 38.18°,44.25°,64.72° and 77.40° confirming the formation of Ag nanoparticles.[13]

Germination Rate

Under uniform experimental conditions, i.e., irrigated conditions and time frame, nanoparticle-coated seeds outperformed in all three setups (Soil, Hydroponics, and Germination sheet) as compared to control seeds in terms of germination rate. The seeds coated with nanoparticles showed improved germination in the following order: NCC, GCC, BCC, NAC, BAC, and GAC. NCC based NPs coated seeds incredibly showed 100% germination in the soil as well as on the germination sheet and 96.67% germination in

Hydroponics; the GCC based NPs showed 100% germination in the soil, 96.67% germination in hydroponics and 96% on germination sheet; the BCC based NPsshowed 96.67% germination in the soil, 100% germination in hydroponics and 93.33% germination on germination sheet.

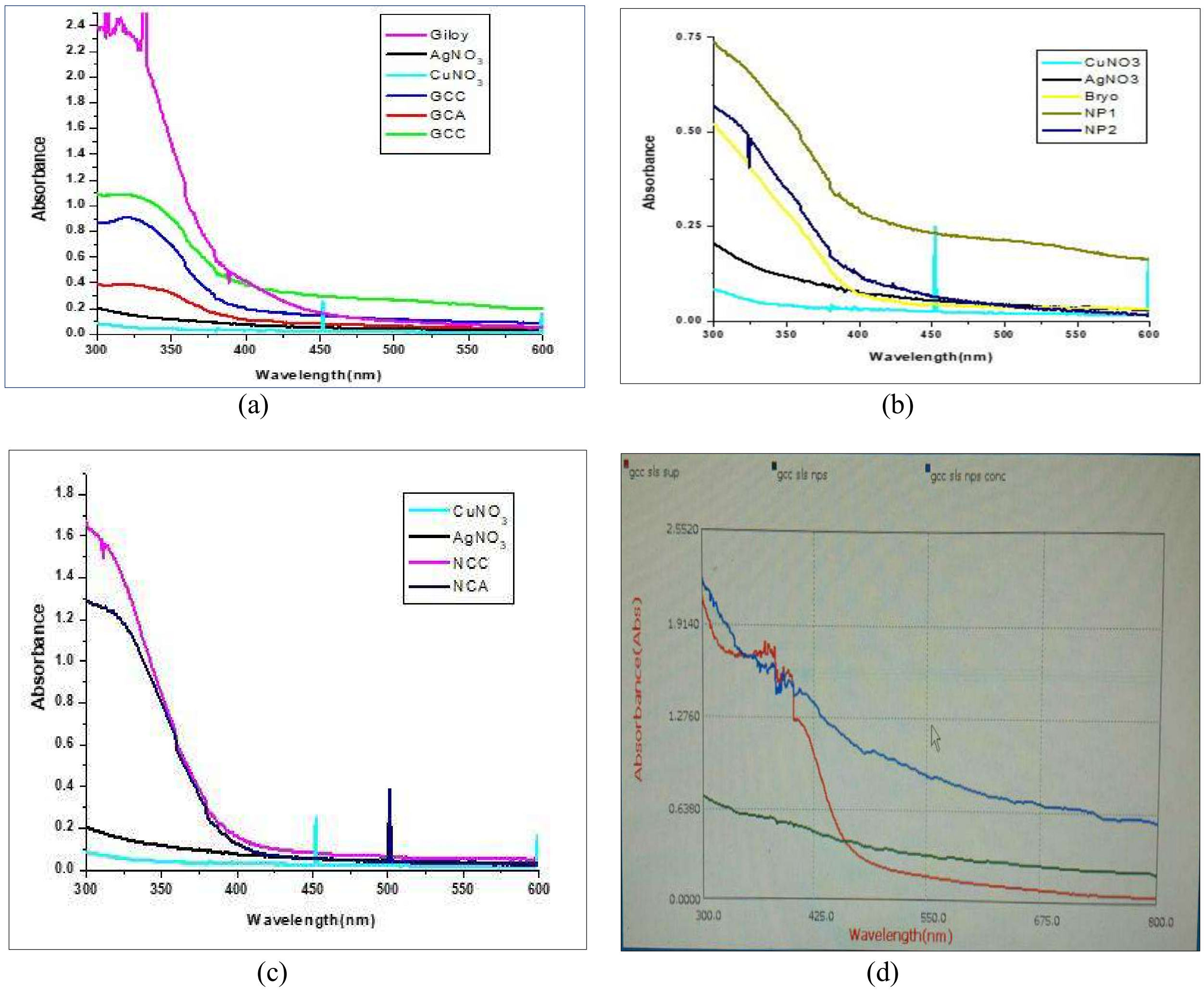

Fig.-1: UV-Visible Spectra for different Nanoparticles, $CuNO_3$, and $AgNO_3$ Solutions. (a) Shows the Spectra of different NPs Formed using GiloyLeaves Extract. Here, the Inset Graph Shows the Peak of GAC AgNPs at 420 nm, Confirming the Formation of AgNPs. (b) shows Absorbance of BryophyllumExtract along with other NPs Formed From this Extract. (c) Shows the Formation of NCC and NCA NPs. (d) Shows the Comparison between GCC Suspension, Synthesised NPs and Concentrated NPs(all having SLS as a Capping Agent in Preparation)

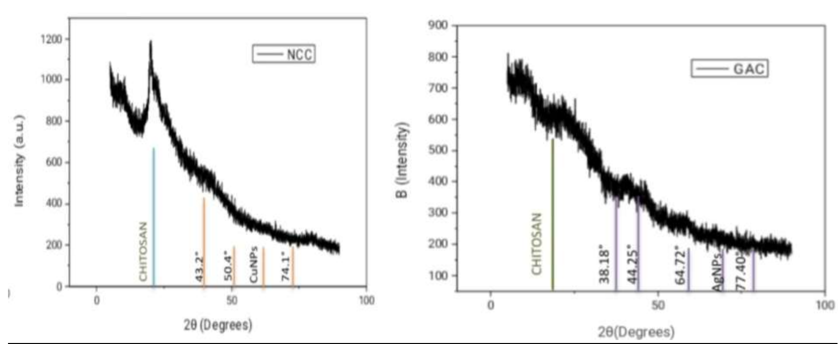

Fig.-2: (a) and (b) Shows the X-ray Diffractogram of GAC (showing peaks of Ag 38.18,44.25,64.72, and 77.40°) and NCC Nanoparticles (showing peaks of Cu at 43.2,50.4, and 74.10°), Respectively

While the control seeds germination percent lies in 73% - 83.33% range in different setups(Fig.-3).Upon germination rate analysis, copper-based nanoparticles demonstrated the most elevated germination rate of *Vigna radiata*. In congruent nanoparticle-based studies, it was observed that AgNPs similarly enhanced the germination of B. *ovalifoliolata* and *E. fistulosum.*[17,18]CuNPs improved the germination of *Triticum aestivum,*[19] and other nanoparticles like FeNPs and SiNPs improved the germination of *Hordeum vulgare* and *Zea mays.*[20] Significant studies of different nanoparticles on varieties of plants proved that nanoparticles are emerging as a beneficial component in improving seed germination rate. Therefore, around 20% fewer seeds would be required for the same yield.

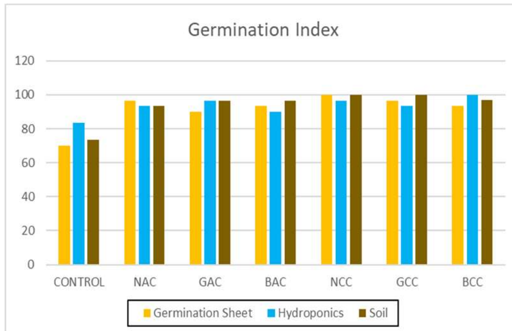

Fig.-3: Graphical Representation of Germination Percentage after 48 hours in Different Setups

Effectiveness of Nanoparticles on Water Management

In the present study, it has been observed that the seeds treated with nanoparticles required less water in comparison to the control. On a daily basis, the amount of water added to each experimental setup was recorded. It was significantly noted that in the germination sheet, control plants consumed 4.2mL more water (Fig.-4) than treated plants, and in the soil also the control plants required 300mL more water (Fig.-5). In order to further explore this nanoparticle attribute, an artificial imposition of drought conditions was induced upon the plants. Withholding water for one day caused wilting in control plants by the following day. Similarly, withholding water for two days leads to some control plants experiencing dieback.In the time of severe water scarcity, it is one of the major concerns to approach sustainable ways to save water, and nanoparticle-based hydrogels are probably one of the best methods to cope with this problem. Nanoparticle-coated seeds having hydrogel retain the water and minimise the evaporation. It leads to sustainable water use, reducing irrigation needs around 15% enabling better moisture retention and seedling vigor under water-scarce conditions. This supports eco-friendly agriculture and ensures higher yields with less irrigation, conserving vital resources.

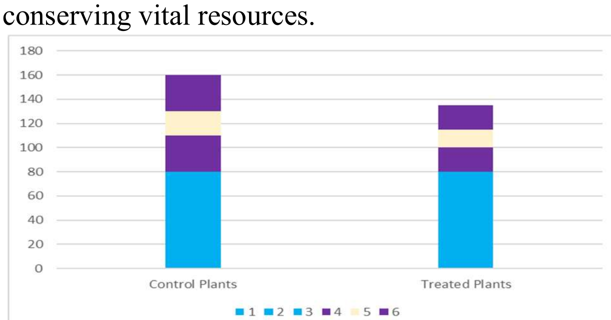

Fig.-4: Graphical Representation of Volume of water (in mL) Poured on the Germination Sheet for 6 Consecutive Days

Phytotoxicity Test

As *Vigna radiata* seeds were treated with laboratory-fabricated foreign particles, there was a risk of toxicity that could affect the growth of the plants. On finding out the germination index of seedlings, the concern could be resolved as a G_{index} value less than hundred (<100) reveals toxicity in plants.[21] The values of different nanoparticle-treated seedlings on the germination sheet and Hydroponics lie in the range of 120.54 to 133.93 and 126.77 to 280.05 (Fig.-6), respectively. Hence, no instances of toxicity were observed in the treated plants, affirming their non-toxic nature in the presence of both metals and diverse leaf extracts.

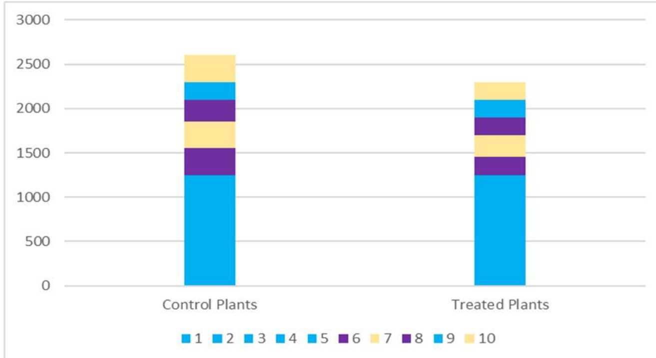

Fig.-5: Graphical Representation of Water Poured in Soil for 10 Consecutive Days

Although there's considerable variation between hydroponics and germination sheet G_{index} values because of the root growth restriction offered by the germination sheet.

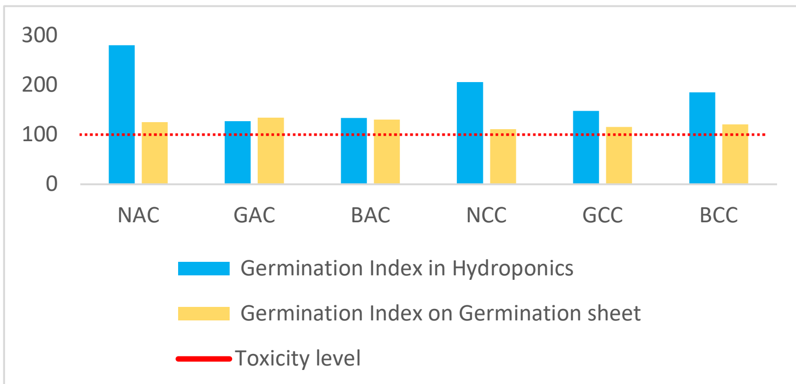

Fig.-6: Graphical Representation of G_{index} Values of Seedlings Coated with different Nanoparticles in Both Hydroponics and Germination Sheets

Morphological Parameters

The stem height (Fig.-7) revealed that there was no consistent pattern of growth in different setups. Statistical analysis of data using a T-test at a significance level of 0.05 also confirmed that there was no significant difference between the control and treated plants' length. Hence, the efficiency of nanoparticles cannot be determined in terms of stem growth.The next important parameter is the root length, directly correlated with the surface area available for water absorption. The root length of plants on the Germination sheet was considered, as during the initial stages of germination, radical growth out of the seed coat is much more prominent.[22] The recorded data (Fig.-8) revealed that the plants subjected to various treatments exhibited enhanced root growth, with a notable emphasis on those treated with BAC-based NPs, which demonstrated moderate growth across all experimental setups, surpassing the standardized control plants. On applying a statistical analysis t-test at a p-value of 0.05, the significant difference between the root lengths was confirmed. The NPs used in the study did not inhibit root growth under the tested conditions. Some of the previous parallel experiments had shown positive as well as negative effects of NPs on root growth, as in *Hordeum vulgare* root was elongated, while in *Lactuca sativa*, it was inhibited by Ag-based nanoparticles.[23]Hence, the selection of nanoparticles should have been judiciously guided by experimental data to prevent any potential inhibition of root growth, which can also signify phytotoxicity.The leaves, as photosynthesis sites, significantly impact plant growth. Although leaf breadth remained almost consistent in the initial days, leaf length exhibited variable growth over days and was considered for the measurement. Many nanoparticles showed better results than the control. BAC-based NPs were best in both setups, exhibited moderate leaf growth, surpassing the leaf length of Control Plants (Fig.-9). On applying a statistical analysis t-test at a p-value of 0.05, the significant difference between the leaf lengths was confirmed.Therefore, the nanoparticles also help in enhancing photosynthesis and ultimately the growth rate.

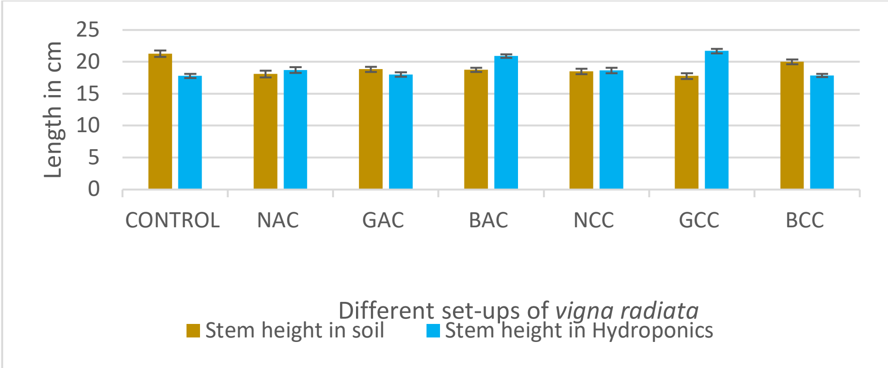

Fig.-7: Graphical Representation of Stem Length of *Vigna radiata* in Soil and Hydroponics after 10 Days of Germination

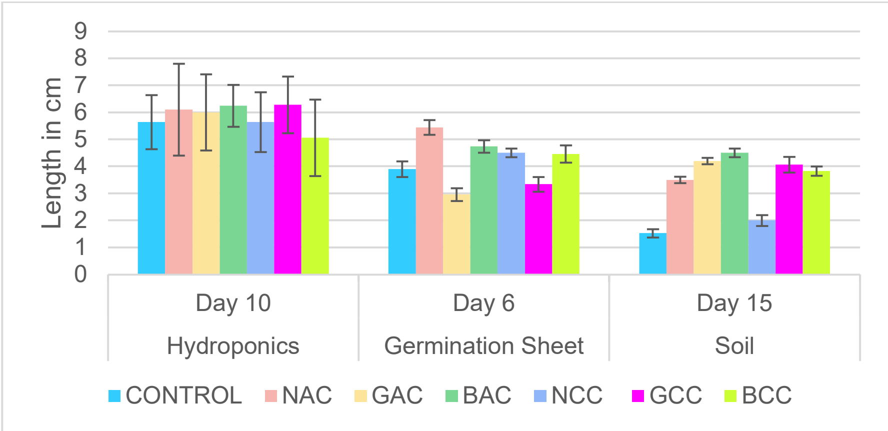

Fig.-8: Graphical Representation of Root Length of *Vigna radiata* in Hydroponics, Germination Sheet, and Soil after a Period of 10 days, 6 days, and 15 days Respectively, of Germination

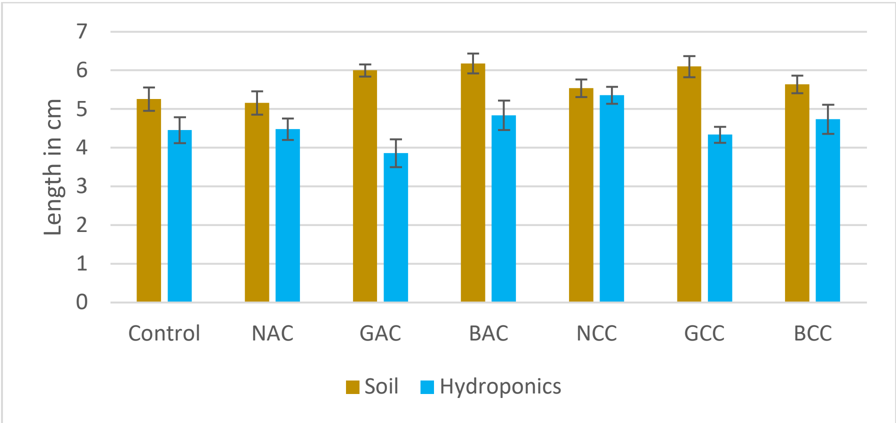

Fig.-9: Graphical Representation of Leaf Length of *Vigna radiata* in Soil and Hydroponics after 10 Days of Germination

CONCLUSION

The conducted study highlights the substantial potential of nanoparticles within the agricultural domain. When considering the life cycle of the plant, germination rate comes first. The results clearly indicate that nanoparticle-treated seeds exhibited markedly improved germination rates relative to untreated controls. The higher the germination rate, the higher the production with minimal seed requirement that would ultimately lead to food security and fulfil SDG 2, i.e., zero hunger.Especially, the Copper-based nanoparticles yielded the most favourable outcomes for *Vigna radiata*. The assessment of morphological parameters also revealed a degree of significant effect of nanoparticles on the growth of roots and leaves. BAC-based NPscan be considered among all other nanoparticles to opt for a moderate growth in the case of *Vigna radiata*. But none of them hamper the growth rate and were proven to be non-toxic to plants, thereby solidifying their eco-friendly nature and hence fulfill the SDG 12, i.e., responsible Consumption and Production,reduce chemical use, promote eco-friendly inputs.

A pivotal result arising from this study was the appreciable reduction in water requirements for nanoparticle-treated plants. This discovery holds significant implications for resource-efficient agricultural practices, as plants grown from treated seeds consumed significantly less water compared to control plants. This was due to the hydrogel nature of synthesised NPs. The ability of nanoparticle-treated plants to withstand artificial drought conditions and exhibit better survival rates highlights their potential for drought tolerance, which can greatly benefit agriculture in water-limited environments. Hence, the use of Nanoparticles is helpful in achieving SDG 6, i.e., clean water and sanitation. The study of different nanoparticles on different plants will surely ensure an eco-friendly revolution in agricultural fields, with practices enhancing growth with less irrigation. Also, the other beneficial aspects are yet to be unveiled. Future recommendations of our research include conducting large-scale field trials, analysing nanoparticle concentrations and their effects in real environmental conditions. Additionally, the study of the mechanism of nano-fertilizer development, their impact, and integration with existing systems is very important. By exploring more of the potential of nanoparticles, we could expand the potential of nanoparticles in agriculture. This can enhance crop yields and water management.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-6: Clean water and Sanitation*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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